

# Fast and Sensitive Determination of Quaternary Amines by UHPLC

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## Overview

**Purpose:** To demonstrate the use of the Thermo Scientific™ Vanquish™ UHPLC to measure low levels of four quaternary amine surfactants of relevance to the biotechnology industry: tetrabutylammonium (TBA) chloride, dodecyltrimethylammonium (DTA) bromide, tetradecyltrimethylammonium (TTA) bromide, and hexadecyltrimethylammonium (CTA) bromide.

**Method:** A rapid gradient elution method using the Vanquish UHPLC system and Corona charged aerosol detection (CAD) for the determination of four quaternary amines is described. Analytes were resolved on a Vanquish C18, 1.5 μm column using an acetic acid/acetonitrile gradient.

**Results:** The four analytes were separated in two minutes. LOQ values varied between 1.7 and 4.8 ng on column (o.c.), with calibration coefficients > 0.9990. Peak area percent relative standard deviations (% RSD) were less than 3.4 for all amounts greater than 6 ng o.c.

## Introduction

In biotechnology, the manipulation of RNA and DNA is often required to isolate them from other complex matrix components. CTA is used both to lyse cell membranes and to solubilize RNA and DNA. CTA in combination with sodium chloride enables the separation of RNA and DNA from polysaccharides.<sup>1</sup> Quaternary amine surfactants are important for biochemical studies: TTA is used in capillary electrophoresis<sup>2,3</sup>; TBA, DTA, have uses in proteomics, phase transfer catalysts, and in the study of surface chemistries. None of these analytes, (see Figure 1 for structures), possess a chromophore, making the use of the Thermo Scientific Vanquish Charged Aerosol Detector necessary for accurate quantitation.

CAD is a mass sensitive technique used with HPLC or UHPLC for the determination of non-volatile and many semi-volatile analytes. CAD is fully gradient compatible, is sensitive to low nanogram levels, has a wide dynamic range and offers consistent response, independent of an analyte's chemical structure. It therefore offers several advantages over other "universal" approaches such as RI and ELSD.

The fast, two-minute analysis of these four quaternary amines was created on the Vanquish UHPLC system, as pictured in Figure 2. Analytes were resolved on a Thermo Scientific™ Vanquish™ Accucore™ C18 column using a rapid water – acetonitrile gradient.

The Corona charged aerosol detector, as shown in the schematic in Figure 3, provides a fast, precise, and quantitative results for both chromophoric and/or non-volatile analytes in a single injection.

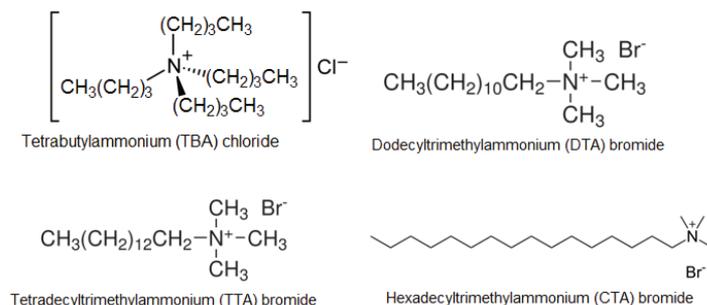


FIGURE 1: Structures of Quaternary Amines.

## Methods

### Liquid Chromatography using the Vanquish binary UHPLC system including:

- Binary Pump H (P/N VH-P10-A)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Charged Aerosol Detector H (P/N VH-D20-A)

Column: Vanquish Accucore C18 column, 1.5  $\mu\text{m}$ , 2.1 x 100 mm (P/N 17101-102130)

Eluent A: Water, 0.1 v/v-% glacial acetic acid

Eluent B: Acetonitrile

Flow Rate: 0.45-0.60 mL/min

Sampler Temperature: Ambient

Injection volume: 2.00  $\mu\text{L}$

Pre-column Temp.: 40  $^{\circ}\text{C}$

Column Temperature: 40  $^{\circ}\text{C}$ , Still Air

Detection: Vanquish Charged Aerosol Detector

Power Function: 1.00

Data Rate: 20 Hz

Filter Constant: 3.6 s

Evaporator Temp.: 60  $^{\circ}\text{C}$

Gas Reg. Mode: Analytical

Gradient:

Time (minutes)	Flow Rate (mL/min)	%B
-1.5	0.50	10
0.0	0.60	10
0.2	0.50	70
0.8	0.47	75
0.9	0.45	100
1.0	0.45	100
1.2	0.50	10
2.0	0.50	10

### Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.2

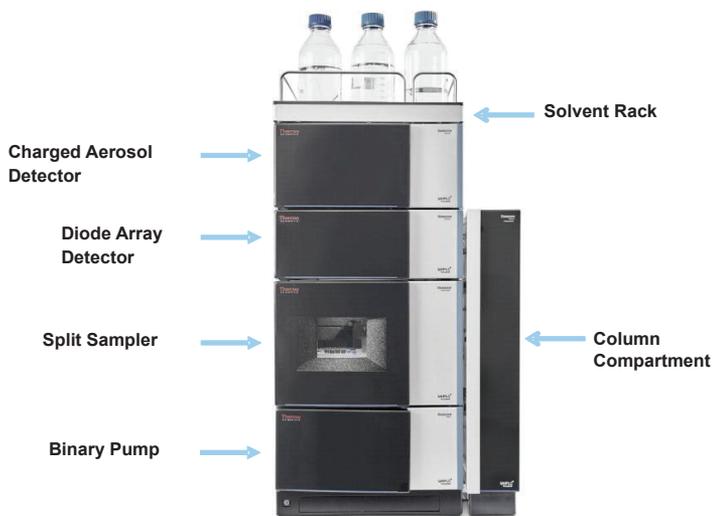


FIGURE 2. Integrated Vanquish UHPLC System with Multi-Modal Analyte Detection.

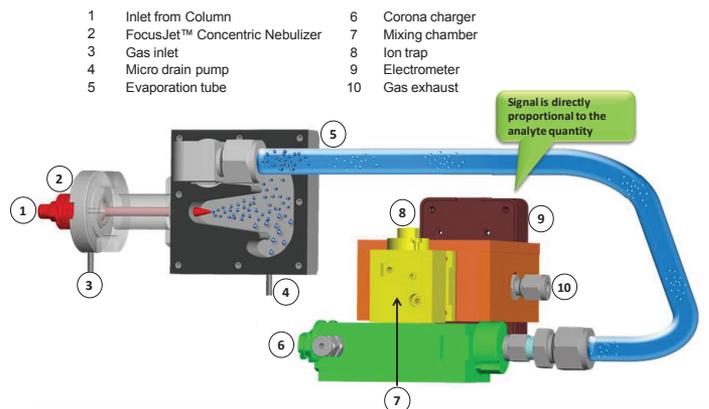


FIGURE 3. Cut-away Diagram of the Charged Aerosol Detector.

## Results

Four quaternary amines, TBA, DTA, TTA, and CTA were analyzed in replicates of five from concentrations of 1.53 – 785 ng o.c. Overlaid chromatograms of five injections at 785 ng o.c. are shown in Figure 4. All peaks were baseline resolved ( $R_s \geq 2.0$ ).

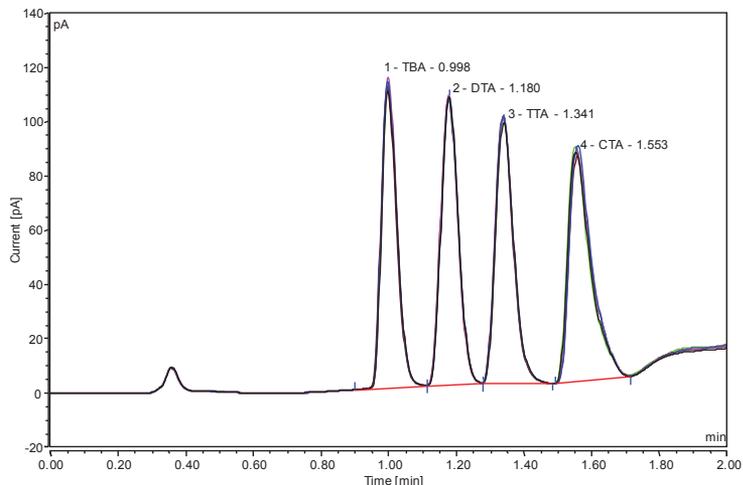
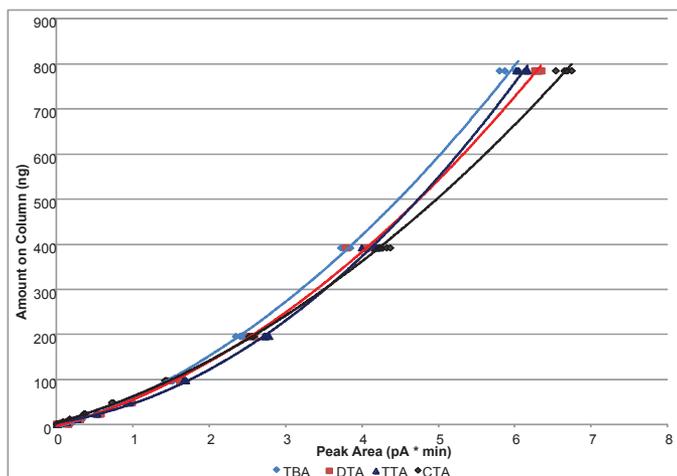


FIGURE 4: Overlaid UHPLC-CAD chromatograms of four quaternary amine surfactants at 785 ng o.c. of the cation, (n=5).

### Calibration and Sensitivity

Five replicate injections were made for each standard solution from 1.53 – 785 ng o.c., using a series of sequential, 50% dilutions. Regression fits using second-order polynomials on inverted axes for the cations TBA, DTA, TTA, and CTA, are shown in Figure 5. The calibration results and sensitivity limits are listed in Table 1. All correlation coefficients,  $R^2$ , were  $\geq 0.9990$ , and the sensitivity limits were estimated using a signal to noise ratios (SNR) of 10 for limit of quantitation (LOQ) and the limit of detection (LOD) was estimated to be half of the LOQ value.



**FIGURE 5:** Regression fits for cations TBA, DTA, TTA, and CTA from 1.53 – 785 ng o.c., n=5.

**TABLE 1:** Calibration and sensitivity results for four quaternary amines.

Parameter	TBA	DTA	TTA	CTA
R <sup>2</sup>	0.9990	0.9992	0.9991	0.9994
Calibration %RSD	5.02	4.54	4.64	3.69
LOQ (ng o.c.)	1.7	2.8	4.8	3.6
LOD (ng o.c.)	0.8	1.4	2.4	1.8

### Accuracy and Precision

Calibration accuracy, as measured by recovery values, is shown in Table 2. The recovery values for all four analytes were within 15 % of the actual value for all amounts injected, between 12.3 and 785 ng o.c., well-encompassing the typical target  $\pm$  50% dynamic range used in many industries.

**Table 2.** Calibration recovery values for four quaternary amines, from 12.3 – 785 ng o.c.

Amount (ng o.c.)	Calibration Recovery Values* (%)			
	TBA	DTA	TTA	CTA
785	100.2	1.02	99.7	99.7
393	98.8	97.3	101.6	101.8
196	100.8	100.6	101.7	99.3
98.1	104.4	107.4	95.9	96.0
49.1	107.5	108.1	88.6	91.4
24.5	106.6	109.2	90.9	93.8
12.3	100.7	94.3	100.9	101.5

Retention time precision with the Vanquish UHPLC system yielded very low relative standard deviation values (RSD). The overall, average retention time RSD was 0.133%, spanning a range of 0.036 – 0.232% for each group of five replicate injections. Peak area precision values are listed in Table 3. Overall, the average peak area RSD value was < 3%, for amounts greater than LOQ.

**Table 3.** Peak area %-RSD values for four quaternary amines, from 3.07 – 785 ng o.c. (n=5).

Amount (ng o.c.)	Residual Standard Deviation for Peak Area (%)			
	TBA	DTA	TTA	CTA
785	1.85	0.47	1.11	1.16
393	1.25	2.97	2.24	1.45
196	1.60	1.04	0.99	0.87
98.1	1.37	0.79	0.83	0.84
49.1	0.81	1.37	1.35	0.91
24.5	2.08	1.43	1.60	1.63
12.3	0.60	1.98	1.04	1.16
6.13	1.64	2.43	3.36	
3.07	12.0	4.26	14.5	

## Discussion

A fast, direct, highly sensitive, precise, and accurate UHPLC-CAD method for the measurement of non-chromophoric cationic surfactants is described. The method has a dynamic range between 3 and 785 ng o.c. for the four quaternary amine cations

Mass recoveries for the four analytes, a measure of accuracy, were all within 12% of targeted amounts from 12.3 – 785 ng o.c. Since the LOQ is below this accuracy range, using a lower level of calibration standards will further improve low-amount accuracy values for this method. Peak area RSD values were all below 3.5% for amounts greater than 6 ng o.c. Although very rapid gradient elution was performed, analyte peak retention time precision was only 0.133% over all injections.

This method is useful for cleaning validations, as well as raw material characterization and purity.

## Conclusions

- The use of the charged aerosol detector provided a sensitive and accurate means for determining low amounts of cationic quaternary amines.
- Since there were no chromophoric analytes, diode array detection (DAD) was not used in this study. However, the combination of both CAD and DAD is a very powerful approach for complete analytical profiling of formulations.<sup>4</sup>

## References

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